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# Interactions of selenium hyperaccumulators and nonaccumulators during cocultivation on seleniferous or nonseleniferous soil – the importance of having good neighbors

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## Summary

- This study investigated how selenium (Se) affects relationships between Se hyperaccumulator and nonaccumulator species, particularly how plants influence their neighbors' Se accumulation and growth.
- Hyperaccumulators *Astragalus bisulcatus* and *Stanleya pinnata* and nonaccumulators *Astragalus drummondii* and *Stanleya elata* were cocultivated on seleniferous or nonseleniferous soil, or on gravel supplied with different selenate concentrations. The plants were analyzed for growth, Se accumulation and Se speciation. Also, root exudates were analyzed for Se concentration.
- The hyperaccumulators showed 2.5-fold better growth on seleniferous than on nonseleniferous soil, and up to fourfold better growth with increasing Se supply; the nonaccumulators showed the opposite results. Both hyperaccumulators and nonaccumulators could affect growth (up to threefold) and Se accumulation (up to sixfold) of neighboring plants. Nonaccumulators *S. elata* and *A. drummondii* accumulated predominantly (88–95%) organic C-Se-C; the remainder was selenate. *S. elata* accumulated relatively more C-Se-C and less selenate when growing adjacent to *S. pinnata*. Both hyperaccumulators released selenocompounds from their roots. *A. bisulcatus* exudate contained predominantly C-Se-C compounds; no speciation data could be obtained for *S. pinnata*.
- Thus, plants can affect Se accumulation in neighbors, and soil Se affects competition and facilitation between plants. This helps to explain why hyperaccumulators are found predominantly on seleniferous soils.

## Introduction

Selenium (Se) is an essential element for humans and animals, but it is toxic at higher concentrations (Terry *et al.*, 2000). There is a narrow margin between Se deficiency and toxicity in animals (Stadtman, 1990). As an essential element, Se is required for the production of selenoproteins, some of which function in scavenging free radicals (Zhang & Gladyshev, 2009). Selenium deficiency may promote cancer and cause other diseases such as white muscle disease, which may be fatal (Cosgrove, 2001). Selenium toxicity is thought to be the result of the similarity of Se to sulfur (S); substitution of S by Se in proteins disrupts protein structure and function (Stadtman, 1990). For plants, Se is also toxic at high concentrations (Anderson, 1993). The essentiality of Se for higher plants is still unproven, but Se is considered a beneficial nutrient for many plant species (Pilon-Smits *et al.*, 2009), perhaps because of better oxidative stress resistance (Cartes *et al.*, 2005; Hartikainen, 2005). Plants readily take up and assimilate Se, a capacity that may be used to alleviate both Se deficiency and toxicity in animals and humans. Plants can be used to clean up excess Se from polluted areas (phytoremediation), and Se-enriched plant material may be considered fortified food (biofortification) (Terry *et al.*, 2000).

Plants mainly take up Se from soil in the form of selenate ($SeO_4^{2-}$), which is taken up inadvertently via sulfate transporters, and metabolized via the S assimilation pathway (for a review see Sors *et al.*, 2005). In this pathway, selenate is reduced to selenite ($SeO_3^{2-}$), which can undergo further reduction to selenide ($Se^{2-}$). This may be incorporated into the organic forms, selenocysteine (SeCys), selenocystathionine (SeCysth) and selenomethionine (SeMet). Plant species differ in their capacity to accumulate Se. While most plant species accumulate Se to concentrations below 100 mg Se $kg^{-1}$ DW, even when growing on Se-rich (seleniferous) soils, some plant species native to seleniferous soils can accumulate Se to concentrations as high as 10 000 mg Se $kg^{-1}$ DW (Beath *et al.*, 1934, 1939; Galeas *et al.*,

2007). These are called Se hyperaccumulators; examples are *Astragalus bisulcatus* (Fabaceae) and *Stanleya pinnata* (Brassicaceae). The Se concentrations in hyperaccumulators are typically 1000-fold higher than those in seleniferous soil, and 100-fold higher than those in other vegetation on the same soil (Galeas *et al.*, 2007). The Se concentrations found in hyperaccumulators would be toxic to other plant species. A clue to the tolerance mechanism of hyperaccumulators was found using microfocused X-ray fluorescence (μXRF) mapping and micro-X-ray absorption near edge structure (μXANES) spectroscopy, which revealed a stark contrast in spatial distribution and chemical speciation of Se between hyperaccumulators and nonaccumulators. While nonhyperaccumulator plants were found to accumulate Se primarily in the leaf vasculature as selenate (de Souza *et al.*, 1998; Freeman *et al.*, 2006a), Se hyperaccumulators accumulated Se predominantly in their leaf epidermis as MeSeCys (Freeman *et al.*, 2006a). Thus, hyperaccumulators avoid Se toxicity by storing Se in peripheral tissues and converting it to methylselenocysteine (MeSeCys), a nonprotein amino acid. The enzyme mediating this conversion is SeCys methyltransferase (SMT; Neuhierl & Böck, 1996).

Since hyperaccumulators are found predominantly on seleniferous soils, they appear to have a physiological or ecological need for Se (Beath *et al.*, 1934). There is ample evidence that Se serves ecological functions for hyperaccumulators. Selenium has been shown to protect plants from a wide variety of invertebrate and vertebrate herbivores, as a result of a combination of deterrence and toxicity (Hurd-Karrer & Poos, 1936; Vickerman *et al.*, 2002; Hanson *et al.*, 2003, 2004; Freeman *et al.*, 2006b, 2007, 2009; Galeas *et al.*, 2008; Quinn *et al.*, 2008, 2010). In addition to herbivores, Se can protect plants from Se-sensitive fungal pathogens (Hanson *et al.*, 2003). Thus, hyperaccumulators may have an ecological dependency on Se for protection from various biotic stresses. In addition to elemental defense, Se may be used by hyperaccumulators for elemental allelopathy. Soil adjacent to hyperaccumulators was 7- to13-fold enriched in Se compared with soil collected > 4 m away from hyperaccumulators (El Mehdawi *et al.*, 2011a,b). Accordingly, neighboring vegetation of hyperaccumulators contained two- to 20-fold elevated Se concentrations compared with plants from the same species growing > 4 m away from hyperaccumulators (El Mehdawi *et al.*, 2011a,b). The higher Se concentrations in neighbors of hyperaccumulators may have an allelopathic effect if they are Se-sensitive. Indeed, the percentage vegetative ground cover was on average 10% lower around hyperaccumulators than around comparable nonaccumulator species (El Mehdawi *et al.*, 2011a). Moreover, soil collected next to hyperaccumulators yielded significantly lower germination and growth of the Se-sensitive model plant *Arabidopsis thaliana*, and higher Se accumulation, than soil collected around nonhyperaccumulator species (El Mehdawi *et al.*, 2011a). Based on controlled experiments using agar medium supplied with different concentrations of Se, the Se concentrations in the soil were high enough to explain the observed inhibitive effect on *A. thaliana* germination (El Mehdawi *et al.*, 2011a).

Interestingly, in some cases, hyperaccumulators can also have a positive effect on their plant neighbors (facilitation), if these neighbors are Se-resistant. In field studies, *Symphyotrichum ericoides* and *Artemisia ludoviciana* were twofold bigger when growing next to hyperaccumulators than when they were growing next to nonaccumulators (El Mehdawi *et al.*, 2011b). This benefit appeared to be, at least in part, the result of enhanced protection from herbivory: *S. ericoides* and *A. ludoviciana* harbored fewer herbivores in the field and exhibited less herbivory. Moreover, when taken to the laboratory and used in controlled herbivory studies with grasshoppers collected from the same field site, the high-Se *S. ericoides* and *A. ludoviciana* plants collected next to hyperaccumulators were eaten less than their low-Se counterparts collected next to nonaccumulators (El Mehdawi *et al.*, 2011b).

Several questions remain regarding the effects of hyperaccumulated Se on plant–plant interactions. First, is the higher Se concentration in soil around hyperaccumulators a result of litter deposition or root exudation, or both? In an earlier study it was found that high-Se litter decomposed readily in a seleniferous habitat, harbored more microbial and micro-arthropod decomposers than low-Se litter, and led to enrichment of the underlying soil with Se (Quinn *et al.*, 2010). Release of Se by hyperaccumulator roots via exudation and turnover has never been tested, but may also be substantial, since hyperaccumulator roots can contain Se concentrations around 0.3% of DW (Galeas *et al.*, 2007). Secondly, are the higher Se concentrations in neighboring plants solely a result of higher total soil Se concentrations, or also the result of different chemical Se speciation in soil around hyperaccumulators (which may affect Se bioavailability), and/or of the presence of Se chelators? Do hyperaccumulators affect the speciation of Se in their local environment, including neighboring plants? Thirdly, how is the competition between hyperaccumulators and nonhyperaccumulators affected by the Se concentration of the soil? In this study we aim to address these questions.

## Materials and Methods

### Soil collection and characterization

Soil was collected in June 2010 from two sites on the West side of Fort Collins, CO, USA: Pine Ridge Natural Area (40°32.70N, 105°07.87W, elevation 1510 m), and Cloudy Pass (40°37.33N, 105°12.38W, elevation 1570 m). Pine Ridge Natural Area is a seleniferous area with soil composed of Se-rich Cretaceous shale. This semi-arid shrubland harbors at least two species of Se-hyperaccumulating plants: *Astragalus bisulcatus* (Hook.) A. Gray and *Stanleya pinnata* (Pursh) Britton (Galeas *et al.*, 2007). Cloudy Pass is a nonseleniferous area 10 miles northwest of Pine Ridge Natural Area and similar in altitude, climate and vegetation except that no Se hyperaccumulators are present. Cloudy Pass does contain the nonhyperaccumulator species *Astragalus drummondii* Douglas ex Hook. Soil samples were collected at both sites from 0 to 5 cm depth to determine soil properties and elemental concentrations. Soil pH and electroconductivity (EC) were determined as described using a saturated soil paste (Soil Survey Laboratory Methods Manual, 2004). Soil

texture was determined as described by Gee & Bauder (1986) using a hydrometer method for sand, silt and clay. Soil organic matter (SOM) was determined using a modification of the Walkley Black method, by means of a Spectronic 20 (Milton Roy Co., Ivyland, PA, USA) at 610 nm (Soltanpour & Workman, 1981). Soil calcium carbonate ($CaCO_3$) was quantified using gravimetric determination from $CO_2$ evolution (Soil Survey Laboratory Methods Manual, 2004). Soil elemental analysis was performed as described in the following.

### Plant material

Seeds from *A. bisulcatus* and *A. drummondii* were obtained from Western Native Seed, Coaldale, CO, USA. *Stanleya pinnata* seeds were collected from seleniferous soil at Pine Ridge Natural Area in Fort Collins, CO. *Stanleya elata* M.E. Jones seeds (accession #113) were collected from nonseleniferous soil in Nevada at N 37°26.699 W 117°21.896, at an elevation of 1515 m.

### Cocultivation experiment on seleniferous and nonseleniferous soils

The soil collected from Pine Ridge Natural Area (seleniferous) and from Cloudy Pass (nonseleniferous) was sieved (1 mm mesh) to remove large stones and organic material, and mixed 3 : 1 with Turface® (Buffalo Grove, IL, USA) to make the aeration adequate and to enhance drainage. A thin layer of course gravel and sand was placed in the bottom of 10 × 10 cm pots, and the soil–Turface mixture placed on top. Each pot was placed on an individual tray to catch leachate and keep it available for the plants.

*Stanleya pinnata* and *S. elata* seeds were surface-sterilized by rinsing for 20 min in 20% bleach, followed by five 10 min rinses in sterile water. The *A. bisulcatus* and *A. drummondii* seeds were first scarified with sand paper and then surface-sterilized. The seeds were germinated on sterilized, wet filter paper under continuous light at 23°C in a plant growth cabinet. The emerging seedlings were carefully transferred to the pots. Two plants were placed in each pot. For each soil type the following seven species combinations were created, using six replicates per treatment: two plants of the same species, from *A. bisulcatus*, *S. pinnata*, *A. drummondii* or *S. elata*; two plants of different species, either one hyperaccumulator and one nonhyperaccumulator (*A. bisulcatus* and *A. drummondii* or *S. pinnata* and *S. elata*); or two hyperaccumulators (*A. bisulcatus* and *S. pinnata*). The plants were watered twice a wk with water and once a wk with 0.5-strength Hoagland solution (Hoagland & Arnon, 1938). After 2 months, when the plants became bigger, they were transferred to 14-cm-diameter round pots. Again, a thin layer of gravel and sand was placed on the bottom, and the area around the transplanted soil was filled up with a similar mixture of soil (from the same source as originally) and Turface. The plants were cultivated for an additional 4 months and then harvested. At harvest, the plants were rinsed, divided into shoot and root, dried, and then measured for shoot and root biomass. At that point shoot and root samples were collected for elemental analysis as described later.

### Cocultivation experiment on Turface supplied with different Se concentrations

Essentially the same experimental outline was followed as described earlier for the soil cocultivation experiments, with the difference that the plants were cultivated in 100% Turface growth medium, and treated once a week with different concentrations of Se (0, 10, 20, 40 or 80 μM $Na_2SeO_4$), and twice a week with 0.5-strength Hoagland solution. Also, five (rather than six) replicates were planted for each of the seven plant species combinations and Se concentration. At harvest, the youngest mature leaf was collected from *A. drummondii* and *S. elata* and immediately flash-frozen using liquid nitrogen for X-ray microprobe analyses as described later.

For root exudate collection, we used a modification of the method described by Cakmak *et al.* (1996). Specifically, plants of all four species were grown on Turface with two plants of the same species per pot ($n$ = 3) and treated with 20 μM $Na_2SeO_4$ as described earlier. The plants were harvested after 6 months, gently washed, and transferred to 50 ml of distilled water. After 2 d the plants were transferred to another container with 50 ml water. After another 3 d, this second volume of water and root-released compounds (which will hereafter be referred to as exudate) was collected and analyzed for Se concentration as described in the following. Furthermore, some of the exudate fractions were frozen for Se speciation as described later. In addition, the exudate fractions were used to extract some Pine Ridge Natural Area soil. To 2 g of soil was added 6 ml of exudate, and after mixing by rotation for 1 h at room temperature, allowed to settle overnight at 4°C. The liquid fraction was then removed and used for elemental analysis and X-ray microprobe analyses as described later.

### Selenium distribution and speciation

Selenium speciation was compared in leaf material of *S. elata* grown next to *S. elata* and *S. elata* grown next to *S. pinnata*, as well as in leaves of *A. drummondii* growing next to *A. drummondii* and *A. drummondii* growing next to *A. bisulcatus*. Root exudates and extract from seleniferous (Pine Ridge) soil collected using these exudates were also analyzed for Se speciation. Selenium distribution and local speciation were determined using μXRF mapping and Se K-edge μXANES spectroscopy, respectively, both as described by (Quinn *et al.* 2011). Owing to the time-intensive nature of μXRF and μXANES studies, one biological replicate was analyzed per treatment; for each of these replicates XANES spectra were collected at three different locations on the sample. Red selenium (white line position set at 12 660 eV) was used to calibrate the spectra. Linear least-squares (LSQ) fitting of XANES spectra was performed in the 12 630–12 850 eV range using a library of standard selenocompounds. The best LSQ fit was obtained for minimum normalized sum-squares residuals: $\mathrm{NSS} = 100 \times \left(\sum(\mu_{exp} - \mu_{fit})^2 / \sum(\mu_{exp})^2\right)$, where μ represents the normalized absorbance. The error on the percentages of species present is estimated to ± 10%. All data processing and analyses were performed with a suite of custom

LabVIEW (National Instruments, Austin, TX, USA) programs available at the beamline.

### Elemental analysis

Entire youngest mature leaves were collected from *A. bisulcatus*, *S. pinnata*, *A. drummondii* and *S. elata* for Se concentration analysis. Samples were rinsed with distilled water to remove any external Se and then dried at 45°C for 48 h. The samples were then digested in nitric acid as described by Zarcinas *et al.* (1987). Soil samples were dried, sieved, and extracted with ammonium bicarbonate-diethylenetriaminepentaacetic acid (AB-DTPA) as described by Soltanpour & Schwab (1977). Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used as described by Fassel (1978) to determine elemental concentrations.

### Statistical analysis

The software JMP-IN (3.2.6, SAS Institute, Cary, NC, USA) was used for statistical data analysis. A Student's *t*-test was used to compare differences between two means. ANOVA (one-way and two-way) followed by a post-hoc Tukey–Kramer test was used when comparing multiple means. In cases where the assumptions underlying these tests (normal distribution, equal variance) were not met, the data were transformed ($\log_{10}$, square root, or reciprocal) and reanalyzed. In one case transformation was not sufficient to meet the assumptions and therefore a non-parametric test was used (Kruskal–Wallis/Wilcoxon). Correlation analysis was used to correlate plant biomass with substrate Se concentration.

## Results

When pairs of plants from the same species were grown together in one pot, the total biomass attained by Se hyperaccumulators *A. bisulcatus* and *S. pinnata* was two- to threefold larger on seleniferous (PR) soil than on nonseleniferous (CP) soil (Fig. 1a; see Table 1 for soil properties). For nonhyperaccumulator species *A. drummondii* and *S. elata*, there was no significant difference in growth on nonseleniferous and seleniferous soil (Fig. 1a). When growth on each soil was compared between the four plant species, there was a pronounced difference between hyperaccumulators and nonaccumulators with respect to their performance on seleniferous soil: the average shoot and root DW of *A. bisulcatus* and *S. pinnata* was two- to fourfold larger than those of *A. drummondii* and *S. elata* (Fig. 1b). On nonseleniferous soil there were no significant differences in growth between hyperaccumulators and nonhyperaccumulators (Fig. 1c).

When two plants from different species, one hyperaccumulator and one nonhyperaccumulator from the same genus, were grown together in one pot on seleniferous soil, the hyperaccumulators were bigger than the nonhyperaccumulators in both cases (Fig. 2a,b). *A. bisulcatus* was two- to threefold larger than *A. drummondii* (Fig. 2a); the root DW of hyperaccumulator *S. pinnata* was twofold larger than that of *S. elata*; the shoot DW

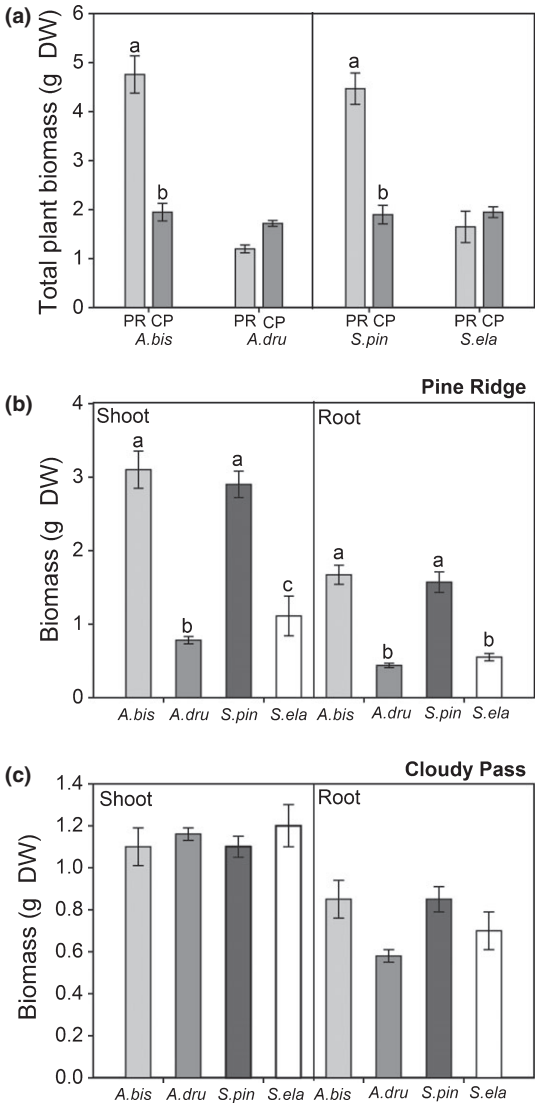



**Fig. 1** Comparison of total plant biomass (a) or shoot and root biomass (g DW) (b, c) between hyperaccumulators *Astragalus bisulcatus* (*A. bis*) and *Stanleya pinnata* (*S. pin*) and nonaccumulators *Astragalus drummondii* (*A. dru*) and *Stanleya elata* (*S. ela*) grown in pots on seleniferous soil from Pine Ridge Natural Area (PR) or nonseleniferous soil from Cloudy Pass (CP). Two plants from the same species were grown per pot. The averages and standard errors of the mean (SE) from six replicates are shown. Different lower-case letters above the bars indicate significantly different means (ANOVA, $\alpha = 0.05$).

was not significantly different ($P = 0.08$, Fig. 2b). Fig. 2(c–f) shows the biomass of each of the four species on seleniferous soil as influenced by which neighbor was in the same pot. The shoot and root biomass of hyperaccumulator *A. bisulcatus* was the same when grown with another *A. bisulcatus* plant as it was when it grown with nonhyperaccumulator *A. drummondii*; however, the *A. bisulcatus* biomass was twofold smaller when grown with hyperaccumulator *S. pinnata* (Fig. 2c). The shoot and root biomass of hyperaccumulator *S. pinnata* was larger when grown with another *S. pinnata* plant than when grown with nonhyperaccumulator *S. elata* or with hyperaccumulator *A. bisulcatus*

**Table 1** Soil properties (0–5 cm depth) at the study sites, Pine Ridge Natural Area and Cloudy Pass, Fort Collins, CO, USA

| | Pine Ridge | Cloudy Pass |
|---|---|---|
| Sample ID | Average ± SE | Average ± SE |
| Texture | Sandy Loam | Sandy Loam |
| pH | 7.57 ± 0.03a | 6.57 ± 0.03b |
| EC (mmhos $cm^{-1}$) | 0.43 ± 0.03a | 0.4 ± 0a |
| SOM (%) | 5.8 ± 0.06a | 4.5 ± 0.06b |
| $CaCO_3$ (%) | 16.25 ± 0.14a | 2.03 ± 0.07b |
| $NO_3$-N (mg $kg^{-1}$) | 2.83 ± 0.03a | 1.23 ± 0.03b |
| Al (mg $kg^{-1}$) | 0.78 ± 0.02a | 1.63 ± 0.09b |
| Ba (mg $kg^{-1}$) | 0.72 ± 0.02a | 1.67 ± 0.03b |
| Ca (mg $kg^{-1}$) | 350.20 ± 1.3a | 324.30 ± 2.3b |
| Cd (mg $kg^{-1}$) | 0.41 ± 0.01a | 0.16 ± 0.01b |
| Cr (mg $kg^{-1}$) | 0.08 ± 0.06a | 0.11 ± 0.05a |
| Cu (mg $kg^{-1}$) | 11.7 ± 0.09a | 4.60 ± 0.12b |
| Fe (mg $kg^{-1}$) | 7.5 ± 0.09a | 39.73 ± 0.12b |
| K (mg $kg^{-1}$) | 453 ± 1.74a | 394 ± 1.53b |
| Mg (mg $kg^{-1}$) | 61.3 ± 0.46a | 149 ± 1.97b |
| Mn (mg $kg^{-1}$) | 11.67 ± 0.09a | 4.57 ± 0.09b |
| Mo (mg $kg^{-1}$) | 0.04 ± 0.01a | 0.01 ± 0b |
| Na (mg $kg^{-1}$) | 24.40 ± 0.25a | 13.97 ± 0.12b |
| Ni (mg $kg^{-1}$) | 1.15 ± 0.02a | 0.42 ± 0.02b |
| P (mg $kg^{-1}$) | 2.1 ± 0.06a | 8.1 ± 0.06b |
| Pb (mg $kg^{-1}$) | 1.6 ± 0.06a | 1.23 ± 0.09b |
| S (mg $kg^{-1}$) | 13.9 ± 0.12a | 15.63 ± 0.12b |
| Se (mg $kg^{-1}$) | 1.7 ± 0.06a | 0.11 ± 0b |
| V (mg $kg^{-1}$) | 1.2 ± 0.06a | 0.15 ± 0b |
| Zn (mg $kg^{-1}$) | 1.63 ± 0.12a | 2.33 ± 0.03b |

Shown are means ± standard error ($n$ = 3).
a and b denote significant differences.

(Fig. 2d). The shoot and root biomass of nonhyperaccumulator *A. drummondii* was twofold lower when grown next to another *A. drummondii* than when grown next to hyperaccumulator *A. bisulcatus* (Fig. 2e). The shoot biomass of nonhyperaccumulator *S. elata* was significantly smaller when grown next to another *S. elata* than when grown next to *S. pinnata*; the root biomass was not significantly different (Fig. 2f).

When a hyperaccumulator and a nonhyperaccumulator from the same genus were cocultivated on nonseleniferous soil, the nonhyperaccumulator *A. drummondii* was twofold bigger than the hyperaccumulator *A. bisulcatus* (Fig. 3a), while the two *Stanleya* species did not show significantly different growth (Fig. 3b, $P$ = 0.136 for shoot and 0.076 for root). Interestingly, the average shoot and root DWs of hyperaccumulator *A. bisulcatus* were twofold higher when grown with hyperaccumulator *S. pinnata* than when grown with another *A. bisulcatus* or with nonhyperaccumulator *A. drummondii* (Fig. 3c). Similarly, there was a pronounced increase in the average shoot and root DWs of hyperaccumulator *S. pinnata* when it was grown with hyperaccumulator *A. bisulcatus* compared with when it was grown with another *S. pinnata* plant or with nonhyperaccumulator *S. elata* (Fig. 3d). Nonhyperaccumulator *A. drummondii* was bigger when grown next to *A. bisulcatus* than when grown next to another *A. drummondii* (Fig. 3e). Nonaccumulator *S. elata* shoots were significantly bigger when growing next to hyperaccumulator *S. pinnata* than when growing next to *S. elata*; there was no difference in root biomass (Fig. 3f).

Two-way ANOVA was used to test whether the relationship between a pair of plants was different in high-Se vs low-Se soil. For *A. bisulcatus* a significant interaction was found for both shoot and root DW ($P$ < 0.0001): *A. bisulcatus* plants that had *S. pinnata* as a neighbor had less biomass when grown on high-Se soil, but more biomass when grown on low-Se soil, as compared with plants that had other neighbors (Figs 2c, 3c). There was also a significant interaction ($P$ < 0.0001) for both shoot and root DW for *S. pinnata*: plants that had *S. pinnata* as a neighbor had more biomass when grown on high-Se soil, but less biomass on low-Se soil compared with plants with other neighbors (Figs 2d, 3d). The effects of neighboring species on the growth of the two nonhyperaccumulator species were less soil-dependent. Only the (positive) effect of *S. pinnata* on root DW of *S. elata* was soil-dependent, namely only observed on low-Se soil.

Fig. 4 shows the Se concentration in the shoots and roots of each of the four species on seleniferous soil, as influenced by which neighbor was in the same pot. The most pronounced neighbor effect was found for hyperaccumulator *S. pinnata*, whose shoot and root Se concentration was eight to 10-fold higher when grown in a pot with nonhyperaccumulator *S. elata* than when grown with another *S. pinnata* or with hyperaccumulator *A. bisulcatus* (Fig. 4b). Furthermore, roots of *A. bisulcatus* contained a lower Se concentration when growing next to *S. pinnata* compared with other species (Fig. 4a), and roots of *A. drummondii* contained a lower Se concentration when growing next to *A. bisulcatus* than when growing next to *A. drummondii* (Fig. 4c). Nonsignificant trends worth noting are that the Se concentration in *A. bisulcatus* was *c.* 50% higher when growing next to another *A. bisulcatus* than when growing next to *A. drummondii* or *S. pinnata* (Fig. 4a), and the Se concentration in *S. pinnata* was *c.* 50% higher when growing next to another *S. pinnata* than when growing next to *A. bisulcatus* (Fig. 4b). As a result of the combined neighbor effects on plant growth and Se concentration, the total shoot Se accumulation per plant, as calculated from the product of shoot Se concentration (Fig. 4) and shoot biomass (Fig. 2), was threefold higher when the hyperaccumulators were grown next to a hyperaccumulator from the same species as compared with a hyperaccumulator of the other species ($P$ = 0.0005 for *A. bisulcatus* and 0.036 for *S. pinnata*). A similar calculation can be done for *S. elata*, whose shoot Se concentration was 50% higher when grown next to *S. pinnata* than *S. elata* (Fig. 4d, $P$ = 0.208); the total shoot Se accumulation per *S. elata* plant was threefold higher next to *S. pinnata* ($P$ = 0.088).

The Se concentrations in shoots and roots of the four species when grown on nonseleniferous soil are shown in Fig. 5. As expected, the Se concentrations were substantially lower than in the plants grown on seleniferous soil, but still measurable. The shoot Se concentration in hyperaccumulator *A. bisulcatus* was two- to threefold higher when growing in the same pot with nonhyperaccumulator *A. drummondii* or with hyperaccumulator *S. pinnata* than when growing with another *A. bisulcatus* plant; there were no differences in root Se concentration (Fig. 5a). The root and shoot Se concentrations in hyperaccumulator *S. pinnata*

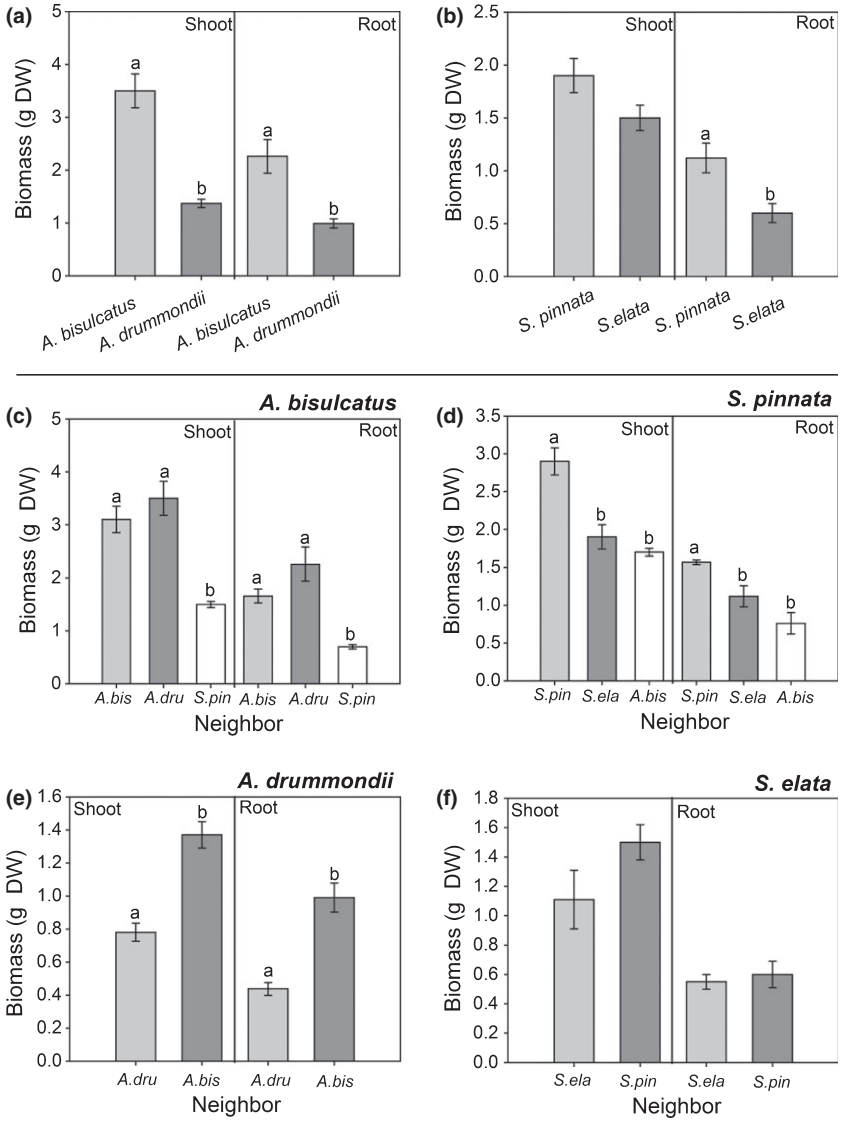



**Fig. 2** Shoot and root biomass (g DW) of hyperaccumulator plants *Astragalus bisulcatus* (*A. bis*) and *Stanleya pinnata* (*S. pin*) and nonaccumulators *Astragalus drummondii* (*A. dru*) and *Stanleya elata* (*S. ela*) grown in pots on seleniferous soil from Pine Ridge Natural Area. Two plants from the same or different species were grown per pot. (a,b) Biomass of each of two neighbors that were cocultivated in one pot. (c–f) Growth of each of the four species as influenced by which neighbor was in the same pot. Values shown are means ± SE ($n$ = 6); different lower-case letters above the bars indicate significantly different means (ANOVA, $\alpha$ = 0.05).

were five to 10-fold higher when grown in same pot with nonhyperaccumulator *S. elata* than when grown with another *S. pinnata* or with hyperaccumulator *A. bisulcatus* (Fig. 5b), which is similar to the results found on seleniferous soil (Fig. 4b). Root Se concentration of nonhyperaccumulator *A. drummondii* was twofold lower when growing in a pot with another *A. drummondii* than when growing with hyperaccumulator *A. bisulcatus* (Fig. 5c). There were no significant differences in shoot Se concentration ($P$ = 0.36, Fig. 5c), or in total Se accumulated per *A. drummondii* shoot, which was 2-2 fold higher when *A. bisulcatus* was the neighbor ($P$ = 0.18). *S. elata* root Se concentrations were about fivefold lower when growing next to *S. elata* than when grown next to *S. pinnata* (Fig. 5d, $P$ < 0.05). The shoot Se concentrations did not differ significantly ($P$ = 0.31), but the total shoot Se accumulation per *S. elata* plant (calculated from the product of Se concentration and biomass) was threefold higher next to *S. pinnata* than when next to *S. elata* ($P$ = 0.066).

The relationship between pairs of plants was in some cases different in high-Se and low-Se soils, as determined by two-way ANOVA. *A. bisulcatus* plants that had *S. pinnata* as a neighbor had lower root Se concentrations than *A. bisulcatus* plant that had other neighbors, but only on high-Se soil (Figs 4c, 5c; $P$ = 0.011). There was also a significant interaction for both shoot and root Se concentrations for *S. pinnata*: having *S. elata* as a neighbor had a significantly bigger positive effect on Se concentration in *S. pinnata* when grown on high-Se soil than when grown on low-Se soil (Figs 4d, 5d; $P$ < 0.001). Furthermore, root Se concentration in *A. drummondii* was affected positively by its neighbor *A. bisulcatus* on low-Se soil, but negatively on high-Se soil ($P$ < 0.01).

To be able to separate the effect of Se on plant–plant interactions from that of other factors (other soil properties, microbial composition), a second cocultivation experiment was carried out using Turface growth medium supplied with different concentrations of $Na_2SeO_4$. When pairs of plants from the same species were grown together in one pot, there was an opposite growth response in Se hyperaccumulators and nonaccumulators. Total plant biomass showed a positive correlation ($P$ < 0.05) with

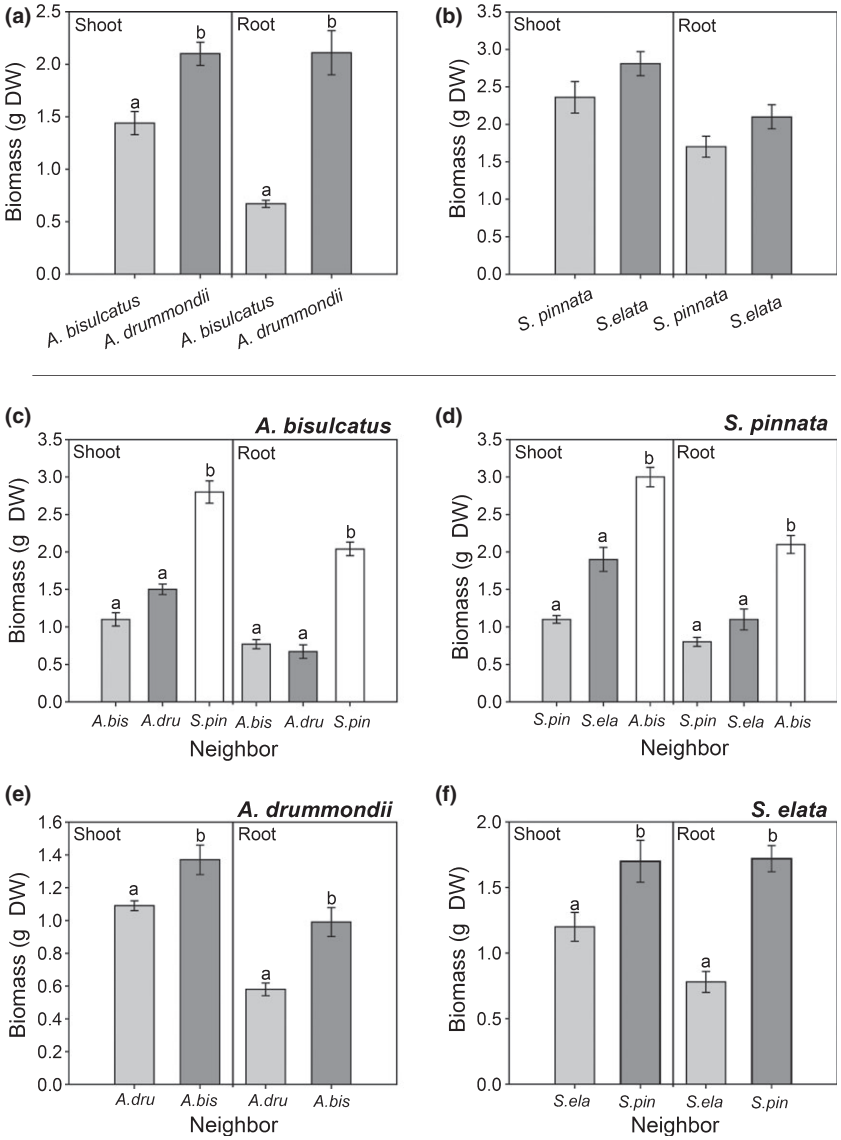



**Fig. 3** Shoot and root biomass (g DW) of hyperaccumulator plants *Astragalus bisulcatus* (*A. bis*) and *Stanleya pinnata* (*S. pin*) and nonaccumulators *Astragalus drummondii* (*A. dru*) and *Stanleya elata* (*S. ela*) grown in pots on nonseleniferous soil from Cloudy Pass. Two plants from the same or different species were grown per pot. (a,b) Biomass of each of two neighbors that were cocultivated in one pot. (c–f) Growth of each of the four species as influenced by which neighbor was in the same pot. Values shown are means ± SE ($n$ = 6); different lower-case letters above the bars indicate significantly different means (ANOVA, $\alpha$ = 0.05).

increasing external Se concentration for hyperaccumulators *A. bisulcatus* and *S. pinnata*, being 4.5-fold and twofold bigger, respectively, when treated with 80 μm $Na_2SeO_4$ than in the absence of Se (Fig 6a,b). By contrast, the biomasses of nonhyperaccumulators *A. drummondii* and *S. elata* decreased six- and 15-fold, respectively, with increasing Se concentration ($P < 0.05$, Fig 6c,d). Thus, the hyperaccumulators were not only Se-tolerant, but even benefited from increasing Se supply, while the nonaccumulators were Se-sensitive, showing 50% growth inhibition at external Se concentrations between 5 and 15 μm.

When two plants from different species, one hyperaccumulator and one nonhyperaccumulator from the same genus, were grown together in one pot, similar growth responses to Se were observed. Hyperaccumulator *A. bisulcatus* had a threefold increase in size with increasing Se treatment, while the cocultivated nonhyperaccumulator *A. drummondii* decreased > 10-fold in size (Fig. 6e). *Stanleya pinnata* had a twofold increase in size with increasing Se supply while nonhyperaccumulator *S. elata* decreased over 60-fold in size (Fig. 6f). As a result of these differential growth responses to Se, the nonaccumulators were bigger than the hyperaccumulators in the absence of Se, while the hyperaccumulators outgrew the nonaccumulators above external Se concentrations of 3 and 8 μm $Na_2SeO_4$, respectively, for the *Astragalus* and *Stanleya* pairs (Fig. 6e,f). When the two hyperaccumulator species were grown together in one pot, their growth responses were also similar to those observed when grown individually: the biomasses of *A. bisulcatus* and *S. pinnata* increased 2.5-fold and fourfold, respectively, with increasing Se supply (Fig. 6g).

Fig. 7(a–d) shows the shoot Se concentration for each of the four species grown on Turface, as influenced by which neighbor was in the same pot. The Se concentration in hyperaccumulator *A. bisulcatus* was twofold higher when growing with nonhyperaccumulator *A. drummondii* than when growing with another *A. bisulcatus* (Fig. 7a). The Se concentration in hyperaccumulator *S. pinnata* was up to 20-fold higher when growing with

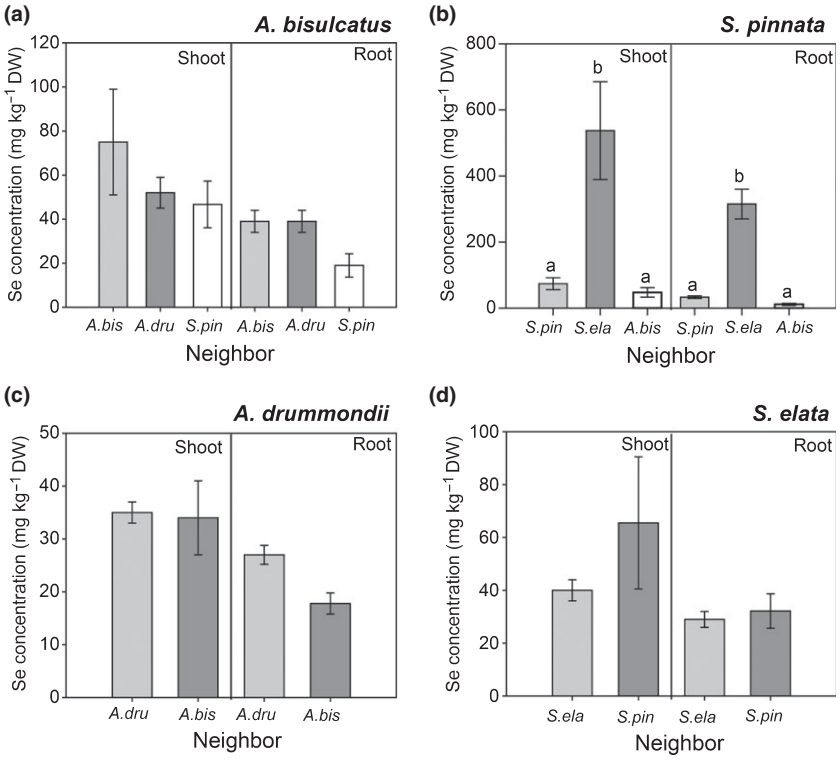



**Fig. 4** Selenium concentration (mg $kg^{-1}$ DW) in shoot and root of hyperaccumulators *Astragalus bisulcatus* (*A. bis*) (a) and *Stanleya pinnata* (*S. pin*) (b) and non-accumulators *Astragalus drummondii* (*A. dru*) (c) and *Stanleya elata* (*S. ela*) (d) after being grown in pots on seleniferous soil from Pine Ridge Natural Area with either another plant from the same species or one from a different species as neighbor. Values shown are means ± SE ($n$ = 6); different lower-case letters above the bars indicate significantly different means (ANOVA, $\alpha$ = 0.05).

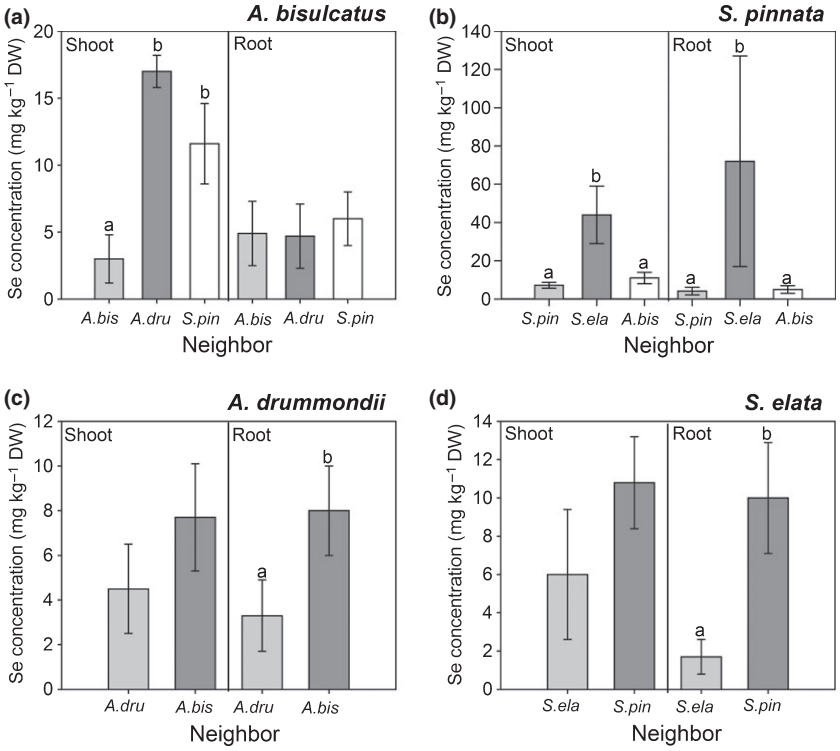



**Fig. 5** Selenium concentration (mg $kg^{-1}$ DW) in shoot and root of hyperaccumulators *Astragalus bisulcatus* (*A. bis*) (a) and *Stanleya pinnata* (*S. pin*) (b) and nonaccumulators *Astragalus drummondii* (*A. dru*) (c) and *Stanleya elata* (*S. ela*) (d) after being grown in pots on nonseleniferous soil from Cloudy Pass with either another plant from the same species or one from a different species as neighbor. Values shown are means ± SE ($n$ = 6); different lower-case letters above the bars indicate significantly different means (ANOVA, $\alpha$ = 0.05).

nonhyperaccumulator *S. elata* than when growing with another *S. pinnata* (Fig. 7b). Nonhyperaccumulators *A. drummondii* and *S. elata* showed increasing tissue Se concentration with increasing Se supply, which was similar in plants grown with a hyperaccumulator or a nonaccumulator neighbor (Fig. 7c,d).

To obtain a better understanding of the mechanism responsible for the observed effects of neighboring plants on plant Se accumulation, root exudate was collected from each of the four species after being grown on Turface and treated with 20 μM $Na_2SeO_4$. The shoot and root Se concentrations in

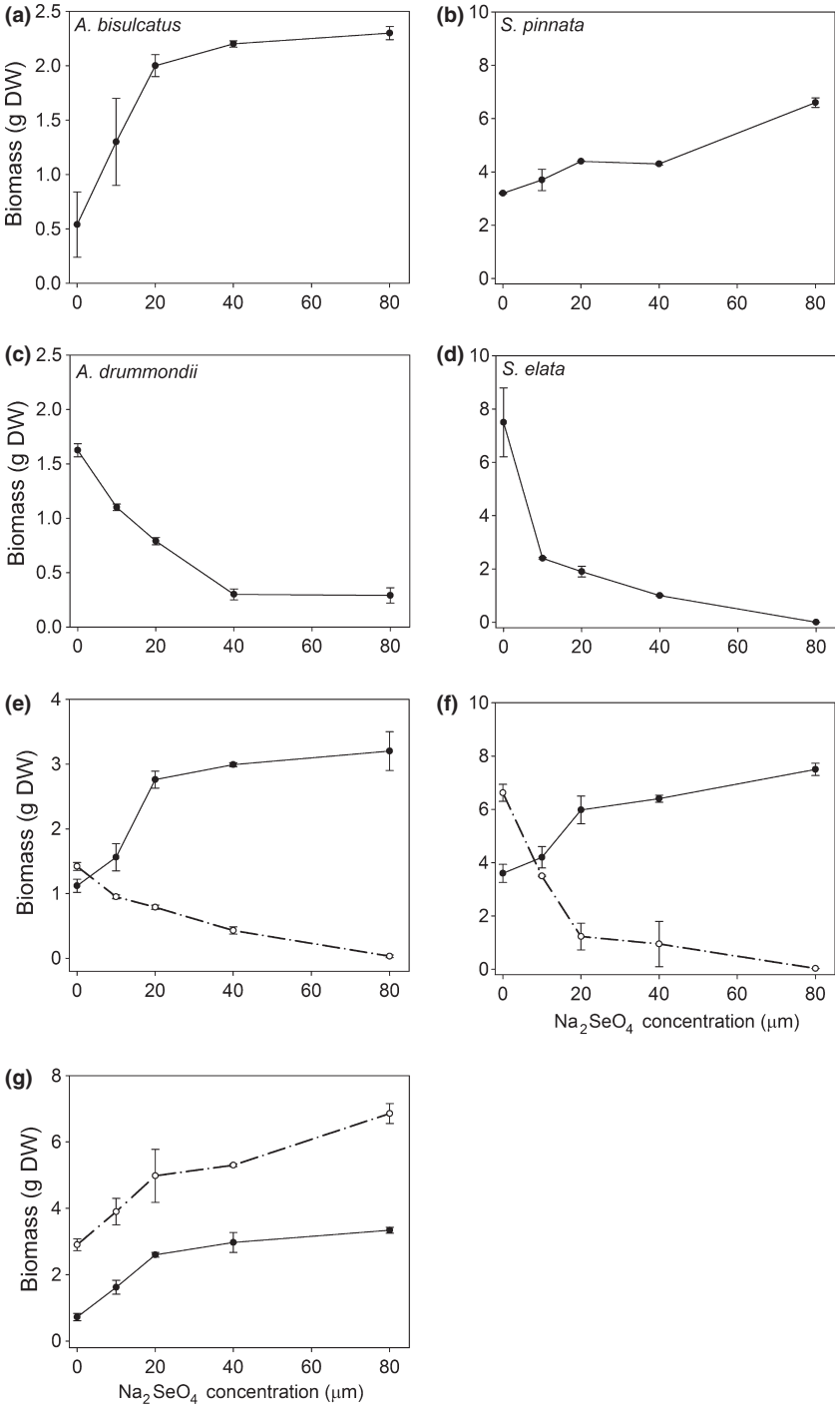
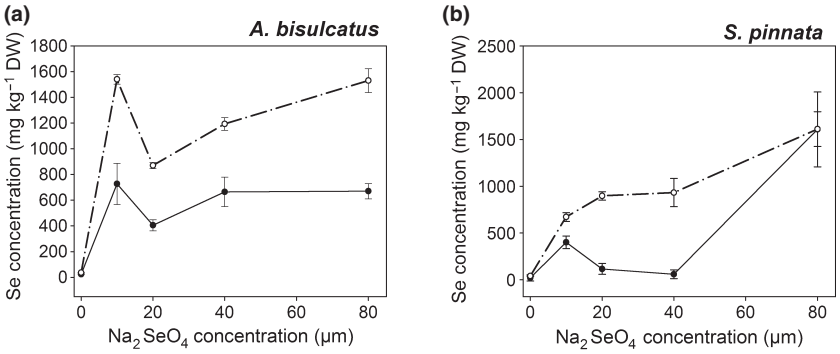



**Fig. 6** Total plant biomass (g DW) of hyperaccumulator plants *Astragalus bisulcatus* and *Stanleya pinnata* and nonaccumulators *Astragalus drummondii* and *Stanleya elata* grown on Turface (gravel) growth medium and treated with different concentrations of $Na_2SeO_4$. (a–d) Two plants from the same species grown in one pot. (e–g) Two plants from different species grown in one pot: (e) *A. bisulcatus* (closed circles) and *A. drummondii* (open circles); (f) *S. pinnata* (closed circles) and *S. elata* (open circles); (g) *A. bisulcatus* (closed circles) and *S. pinnata* (open circles). Values shown are means ± SE ($n$ = 5); different lower-case letters above bars indicate significantly different means (ANOVA, $\alpha$ = 0.05).

hyperaccumulators *A. bisulcatus* and *S. pinnata* were, on average, two- to threefold higher than those in nonhyperaccumulators *A. drummondii* and *S. elata*, but because of the low number of replicates ($n$ = 3) there were few significant differences (Fig 8a,b; note that the $P$ values for the comparison of *A. bisulcatus* and *A. drummondii* shoot and root Se concentrations were 0.087 and 0.165, respectively). The Se concentrations in root exudates were about sixfold higher for the two hyperaccumulators than for the two nonaccumulators (Fig. 8c); here it is worth noting that the hyperaccumulator plants were two- to threefold larger than the nonaccumulators (Fig. 6a–d), so expressed on an equal biomass basis the hyperaccumulators exuded *c.* two- to threefold more Se. Surprisingly, when these root exudates were used to extract seleniferous (Pine Ridge) soil, the extract obtained using hyperaccumulator-derived exudates contained *c.* twofold lower Se concentrations than extract obtained using nonhyperaccumulator exudates (Fig. 8d). After interacting with the seleniferous soil, the hyperaccumulator exudates had decreased in Se concentration while the nonaccumulator exudates had increased in Se.



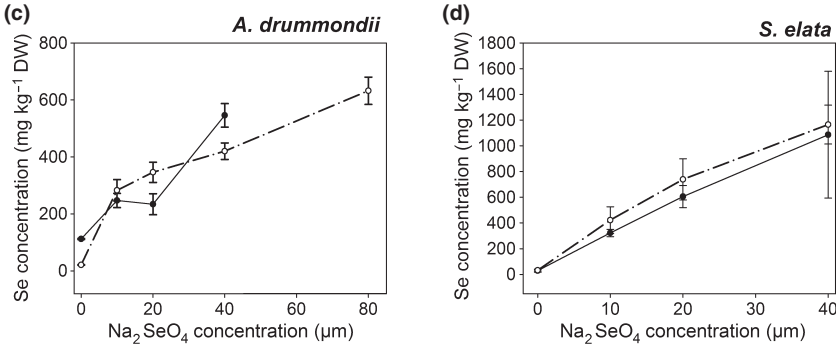
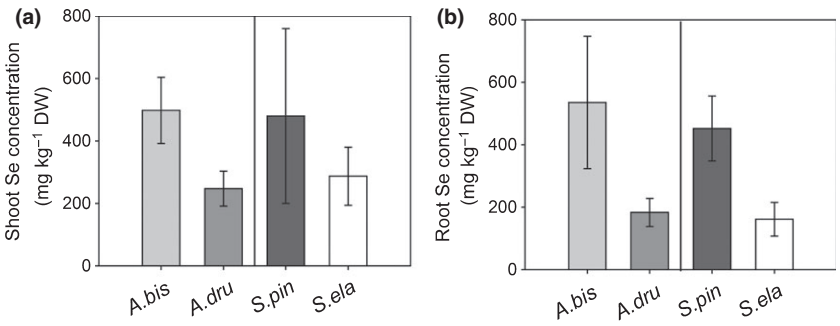




**Fig. 7** Shoot Se concentration (mg $kg^{-1}$ DW) in hyperaccumulators *Astragalus bisulcatus* (a) and *Stanleya pinnata* (b) and nonaccumulators *Astragalus drummondii* (c) and *Stanleya elata* (d) grown on Turface (gravel) growth medium supplied with different concentrations of $Na_2SeO_4$. Two plants were grown per pot, either from the same species (closed circles) or different species from the same genus (*A. bisulcatus* with *A. drummondii* or *S. pinnata* with *S. elata*, open circles). Values shown are means ± SE ($n$ = 5); different lower-case letters above the bars indicate significantly different means (ANOVA, $\alpha$ = 0.05). Note that in some cases no data are shown for the 80 μm treatment for the nonaccumulators because there was not enough plant material.



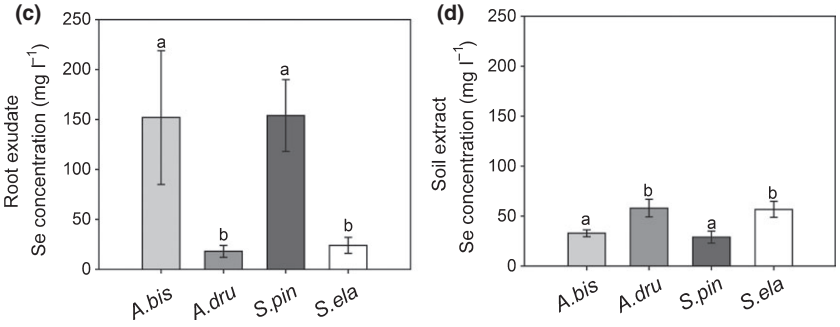




**Fig. 8** (a, b) Selenium concentration (mg $kg^{-1}$ DW) in shoot and root of hyperaccumulators *Astragalus bisulcatus* (*A. bis*) and *Stanleya pinnata* (*S. pin*) and nonaccumulators *Astragalus drummondii* (*A. dru*) and *Stanleya elata* (*S.ela*) grown in pots on Turface (gravel) growth medium supplied with 20 μm selenate, used for collection of root exudate. (c, d) Se concentration in root exudate and in Pine Ridge soil extract obtained using this exudate. Values shown are means ± SE ($n$ = 6); different lower-case letters above the bars indicate significantly different means (ANOVA, $\alpha$ = 0.05).

In addition to affecting the total Se concentration in neighboring plants, it is also feasible that plants can affect their neighbor's Se speciation (i.e. the chemical composition of the selenocompounds). To investigate the Se speciation in nonhyperaccumulator species *A. drummondii* and *S. elata* as affected by their neighbor in the same pot, Se K-edge XANES spectra were collected in leaves of plants grown on Turface and treated with 20 μM $Na_2SeO_4$ (Table 2). The Se in both nonaccumulators consisted primarily (89–95%) of an organic C-Se-C compound, indistinguishable from the standards selenomethionine and methyl-selenocysteine; the remainder was selenate ($SeO_4^{2-}$) (Table 2). The relative abundance of C-Se-C and selenate were similar in *A. drummondii* leaves collected from plants growing next to *A. drummondii* or growing next to *A. bisulcatus* (Table 2). However, speciation in *S. elata* leaves was different when its neighbor was *S. elata* than when its neighbor was *S. pinnata*. *S. elata* that was grown next to *S. pinnata* showed a 3.5-fold lower ($P$ < 0.01) selenate fraction and a concomitant

**Table 2** Selenium speciation in plant leaf material and root exudates determined from X-ray absorption near edge structure (XANES) linear least-squares (LSQ) fitting

| | NSS ($\times 10^{-4}$) | C-Se-C% | $SeO_4^{2-}$% | $SeO_3^{2-}$% |
|---|---|---|---|---|
| *Astragalus drummondii* grown next to *A. drummondii* | | | | |
| Spot 1 | 7.8 | 95.8 | 5.8 | ND |
| Spot 2 | 10.9 | 96.0 | 5.9 | ND |
| Spot 3 | 8.9 | 90.0 | 8.9 | ND |
| Average ± SE | | 93.9 ± 1.9 | 6.8 ± 1.0 | ND |
| *A. drummondii* grown next to *Astragalus bisulcatus* | | | | |
| Spot 1 | 5.1 | 94.9 | 6.2 | ND |
| Spot 2 | 5.4 | 93.9 | 6.9 | 0.4 |
| Spot 3 | 4.5 | 90.8 | 9.5 | ND |
| Average ± SE | | 93.2 ± 1.0 | 7.5 ± 1.0 | ND |
| *Stanleya elata* grown next to *S. elata* | | | | |
| Spot 1 | 5.1 | 88.7 | 9.8 | ND |
| Spot 2 | 4.0 | 89.7 | 11.0 | ND |
| Spot 3 | 4.7 | 88.3 | 11.9 | ND |
| Average ± SE | | 88.9 ± 0.3 | 10.9 ± 0.6 | ND |
| *S. elata* grown next to *Stanleya pinnata* | | | | |
| Spot 1 | 3.5 | 94.0 | 3.7 | ND |
| Spot 2 | 4.0 | 95.4 | 3.5 | ND |
| Spot 3 | 3.4 | 96.4 | 2.4 | ND |
| Average ± SE | | 95.2 ± 0.6 | 3.2 ± 0.6 | ND |
| *A. bisulcatus* root exudate | | | | |
| Spot 1 | 25.6 | 70.7 | ND | 33.5 |
| Spot 2 | 6.4 | 90.6 | ND | 11.9 |
| Spot 3 | 11.6 | 96.7 | ND | 4.5 |
| Average ± SE | | 86 ± 8.0 | ND | 16.6 ± 8.8 |

Plants were grown in pairs on Turface (gravel) and supplied with selenate. One plant per treatment was analyzed and three spectra were obtained from each plant. Values shown for each form of Se represent the % of total Se. The best LSQ fit was obtained for minimum normalized sum-squares residuals (NSS), $NSS = 100 \times \left(\sum(\mu_{exp} - \mu_{fit})^2 / \sum(\mu_{exp})^2\right)$ where $\mu$ represents the normalized absorbance. ND, not detectable; C-Se-C, MeSeCys/SeMet/SeCystathionine (indistinguishable). Forms of Se that were not detected in any of the samples and therefore not tabulated: red and gray $Se^0$, Se-cysteine, Se-cystine, $Se(GSH)_2$.

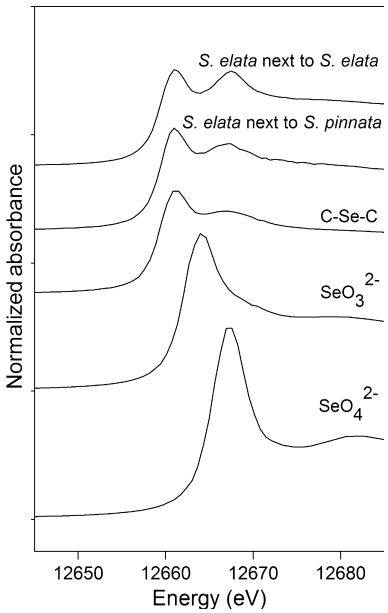



**Fig. 9** Selenium K-edge μXANES (micro-X-ray absorption near edge structure) spectra obtained from leaves of *Stanleya elata* grown in Turface (gravel) growth medium supplied with selenate. The top two spectra are: *S. elata* grown next to another *S. elata*; and *S. elata* grown next to *Stanleya pinnata*. The bottom three spectra are selenocompounds selenomethionine, selenite and selenate, respectively. Note that the SeMet spectrum is virtually indistinguishable from that of another C-Se-C compound, MeSeCys (not shown).

increase in C-Se-C abundance ($P < 0.001$) compared with *S. elata* grown next to another *S. elata* (Fig. 9, Table 2). The Se speciation in the root exudates and soil extracts obtained using root exudates was also analyzed by XANES. Only the *A. bisulcatus* exudate provided useful Se spectra; the main selenocompound (83%) in the exudate was organic Se of a C-Se-C type, and the remainder was selenite (Table 2).

## Discussion

The finding that Se hyperaccumulators perform better on seleniferous soil may indicate that they benefit physiologically from Se. Indeed, the Turface experiment showed that the two hyperaccumulators grew several-fold better with increasing Se concentration, suggesting that the Se in the seleniferous soil was responsible for the better growth of the hyperaccumulators. The beneficial effect of Se on hyperaccumulator growth was previously described by Shrift (1969). A possible mechanism may be enhanced antioxidant activity, as was found for nonhyperaccumulator species (Cartes *et al.*, 2005; Hartikainen, 2005).

In the Turface experiment the growth of the nonaccumulators was worse when Se supply increased, reaching 50% inhibition at *c.* 10–20 μM sodium selenate (0.8–1.6 ppm Se), corresponding with a tissue Se concentration of *c.* 200–250 mg $kg^{-1}$ DW. This is similar to what was found earlier for *Arabidopsis thaliana* (El Mehdawi *et al.*, 2011a). However, the form of Se in *A. drummondii* and *S. elata* was mainly organic C-Se-C (89–95%), while other nonaccumulator species, including *A. thaliana*, accumulate mainly selenate with a minor fraction of C-Se-C (de Souza *et al.*, 1998; Van Hoewyk *et al.*, 2005; Freeman *et al.*, 2006a). Based on XANES data alone the C-Se-C compound in *A. drummondii* and *S. elata* could be MeSeCys, SeMet or SeCysth; these cannot be distinguished. MeSeCys was found earlier to be the predominant form of Se in hyperaccumulators *S. pinnata* and *A. bisulcatus*, which explains their Se tolerance, since MeSeCys does not enter proteins. The intermediate Se accumulator *Stanleya albescens*, on the other hand, accumulated mainly SeCysth and was fairly Se-sensitive (Freeman *et al.*, 2010). The Se sensitivity in *A. drummondii* and *S. elata* could be a result of the accumulation of the more toxic forms SeMet or SeCysth, or of the fact that the remainder of their Se was selenate (4–11%). This form of Se is toxic when accumulated, as a result of pro-oxidant activity (Grant *et al.*, 2011).

The opposite growth responses to Se may affect competition between hyperaccumulators and nonaccumulators: the two likely have different competitive strength depending on soil Se concentrations. When cocultivated in Turface at different Se

concentrations, the threshold above which the hyperaccumulators started to outcompete the nonaccumulators was *c.* 5 μM sodium selenate (*c.* 0.4 ppm Se). In seleniferous soil the Se concentrations are often above this threshold (e.g. in the Pine Ridge soil used here the concentration was 1.5 ppm bioavailable Se), allowing hyperaccumulators to grow well and thus be relatively competitive. The fact that hyperaccumulator growth is impaired in the absence of Se may explain why we find hyperaccumulators primarily on seleniferous soil. In addition to the physiological benefits observed here, hyperaccumulators have already been found earlier to derive ecological benefits from Se accumulation in the form of herbivory and pathogen protection, and allelopathic effects on Se-sensitive plant neighbors. Thus, the hyperaccumulators may also have an ecological dependency on Se for their negative biotic interactions. During the evolution of Se hyperaccumulation, any or all of these physiological and ecological benefits may have played a role as selective pressures.

In addition to the growth responses of individual plant species to Se, it was observed here that plants may affect their neighboring plants' growth and Se accumulation. Both hyperaccumulator and nonaccumulator species could affect their neighbor in terms of growth (up to threefold) and/or Se accumulation (up to sixfold). The biggest effect was observed for *S. elata*, which appeared to enhance the shoot and root Se concentrations in neighboring *S. pinnata* plants three- to sixfold. This was found on seleniferous soil, on nonseleniferous soil, as well as in Turface. The mechanism for this positive effect is not readily apparent. *S. elata* roots were shown to release some Se, but these concentrations were much lower than the Se release from hyperaccumulators. It was interesting, however, that the *S. elata* exudate extracted twofold more Se from seleniferous soil than the *S. pinnata* exudate. Thus, *S. elata* exudate may somehow enhance Se bioavailability for *S. pinnata*. The mechanism is not clear but could, for instance, involve a Se chelator.

The positive effect (threefold) of *S. pinnata* on total shoot Se accumulation in *S. elata* on both soils ($P < 0.1$) might be caused by root Se release, as observed in *S. pinnata* exudate. On Turface there was no effect of *S. pinnata* on *S. elata* growth or Se accumulation. However, *S. pinnata* did seem to affect Se speciation in *S. elata*: *S. elata* contained relatively more organic Se when its neighbor was *S. pinnata* than when it was another *S. elata*. This may be the result of root release of organic Se by *S. pinnata*. In support of this hypothesis, *S. pinnata* roots were shown here to exude significant concentrations of Se, and the form of Se in roots of *S. pinnata* was shown recently to be C-Se-C (Lindblom *et al.*, 2011). While the exudate of *S. pinnata* did not have a strong enough Se signal to obtain reliable speciation information from XANES, *A. bisulcatus* exudate was shown by XANES to contain predominantly C-Se-C. Thus, hyperaccumulators may exude organic Se and, since the main form of bioavailable Se in soil is thought to be inorganic selenate, the root release of organic Se may affect local Se speciation, and with that, Se bioavailability and Se uptake and speciation by neighboring plants. Enhanced bioavailability of Se around hyperaccumulators was also suggested by the earlier finding that, while the soil around hyperaccumulators was seven- to 13-fold enriched with Se, the neighboring plants were enriched up to 20-fold (El Mehdawi *et al.*, 2011b). The finding that hyperaccumulators release Se from their roots supports the hypothesis that hyperaccumulators can phytoenrich their surrounding soil with Se, and that root release of Se is one of the mechanisms for phytoenrichment. Litter deposition and decomposition likely is another mechanism, as indicated by an earlier study (Quinn *et al.*, 2010).

Hyperaccumulator *A. bisulcatus* had a positive effect on growth of nonaccumulator *A. drummondii* on both soils. The neighbor effects between the *Astragalus* species with respect to Se accumulation varied. On nonseleniferous soil where Se concentrations were very low, *A. bisulcatus* plants growing next to *A. drummondii* contained fivefold higher Se concentrations than *A. bisulcatus* plants growing next to *A. bisulcatus*; *A. drummondii* also contained elevated Se concentrations when growing next to *A. bisulcatus* compared with another *A. drummondii*. No such effects were seen on seleniferous soil, but on Turface *A. drummondii* also appeared to stimulate Se accumulation in *A. bisulcatus* at all Se concentrations. The positive effect of *A. drummondii* on Se uptake by *A. bisulcatus* when external Se concentrations were low may be a result of the release of Se chelators by *A. drummondii* roots, whose presence is suggested from the observation that *A. drummondii* exudate did not contain much Se but released more Se from soil than *A. bisulatus* exudate.

The two hyperaccumulators *A. bisulcatus* and *S. pinnata* affected each other's growth and Se accumulation negatively on seleniferous soil, but positively on nonseleniferous soil. This opposite effect may be a result of the 17-fold different Se concentrations, as well other soil properties. Different elements may limit hyperaccumulator growth on the two soils, and the limiting factors may be different for the two hyperaccumulator species. Iron and phosphorous concentrations were four- to fivefold lower in Pine Ridge soil, while nitrate concentrations were twofold higher than in Cloudy Pass soil (Table 1); these elements are often limiting for plant growth, and therefore may have affected plant growth and competition. Furthermore, the Pine Ridge soil was slightly basic while the Cloudy Pass soil was slightly acidic, and soil organic matter was somewhat higher at Pine Ridge, which may have further affected nutrient concentration and bioavailability. In addition to affecting plants directly, these soil properties may affect plant–plant and plant–microbe interactions, for instance by affecting the bioavailability of exuded selenocompounds or Se chelators. Based on the limited information available, we can only speculate about which factors may have caused the opposite effects of the hyperaccumulators on each other. If on Pine Ridge soil Se would be limiting hyperaccumulator growth, and the two species use different mechanisms (e.g. different chelators) to make the soil Se bioavailable, then two plants of the same species may facilitate each other's growth by working together in making the Se bioavailable (as indicated by the higher plant Se concentrations), while two plants of different species make the limited Se less available for the other species. On the nonseleniferous soil, Se may not play a role in plant competition and other elements such as N, Fe, P or K may be limiting plant growth. If the two different hyperaccumulator species are limited by different elements, two plants of the same

species may impede each other's growth by competing for the same limiting nutrient, while two plants of different species may be more complementary in their nutrient requirements, and therefore inhibit each other's growth to a lesser degree.

These studies provide better understanding of the role of Se in plant–plant interactions, particularly between hyperaccumulators and nonaccumulators. Hyperaccumulator species perform better on seleniferous than on nonseleniferous soil, and in general grow better with increasing Se supply. Growth of nonaccumulator species gets worse with increasing Se concentrations. It appears that both hyperaccumulators and nonaccumulators can affect the growth and Se accumulation of neighboring plants. Roots of hyperaccumulators can exude significant concentrations of Se, mainly in organic form, which may lead to higher fractions of organic Se in nonaccumulator neighbors. Nonaccumulators, on the other hand, may be able to enhance soil Se bioavailability, and, with that, Se concentrations in their neighbors. These results are of significance since they offer an insight into how Se affects competition and facilitation between plants, and why hyperaccumulators are found almost exclusively on seleniferous soils.

## Acknowledgements

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